

*Anomalous Liesegang Stratifications produced by the Action of Light.*

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(PLATE 8.)

The investigation to be described in the present paper was originally begun with the intention of studying the Liesegang phenomenon under conditions more strictly defined than have been observed hitherto, and more particularly with definite volume ratios of the reacting solutions. In all the experiments recorded in the literature the amount of solution placed on a given, or unknown, quantity of gel has been arbitrary and is generally not even stated. A quantitative treatment of the phenomenon is impossible *a priori* as long as this ratio is unknown, and would probably become simplest if one, or both, volumes were made—practically—infinite. In the course of the preliminary studies for such experiments an extremely striking anomaly, not observed so far, was discovered, which appears to deserve being put on record before the main investigation is completed.

The reaction chosen for this work was the formation of lead chromate in agar gel by the interaction of lead acetate (dissolved in the gel) with potassium or sodium dichromate, or with potassium chromate. This reaction, in very low concentrations, produces extremely fine and numerous stratifications, which were first obtained by L. J. de Whalley;\* the low concentrations and the cheapness of the ingredients also made it eminently suitable for work involving the use of large volumes. Soon after its discovery the reaction was employed by the author and the results discussed in their bearing on some controversial points, in the paper quoted above. A normal specimen is illustrated in fig. 1 (Plate 8); in this, as in all other specimens photographed, the excess of soluble dichromate present in the gel has been removed by prolonged diffusion, so as to show the lead chromate strata as distinctly as possible.

No anomalies whatever were observed in any of the specimens obtained in 1914. When work on the present investigation was begun, the difficulty generally encountered in the study of the Liesegang phenomenon, viz., that of duplicating results with different brands of raw material, was experienced. This difficulty is generally much more acute with gelatin than with agar, but in the present case one brand of (shred) agar was found to give no strata at

\* Hatschek, 'Koll.-Zeitschr.,' vol. 14, p. 115 (1914).

all, while another (powdered) gave results much inferior to those obtained in 1914. A very satisfactory sample of powdered agar was eventually found and a standard procedure of preparing the 1 per cent. gel used throughout was adopted and rigidly adhered to. The agar sol, cooled to about  $90^{\circ}$ , received an addition of 0.1 gram. of crystallised lead acetate ( $\text{PbAcet}_2 \cdot 3\text{H}_2\text{O}$ ) to every 100 c.c. of sol, and was poured into clean and carefully dried test-tubes. When the gel had cooled to room temperature, a solution containing 5 gram. per litre of crystallised potassium or sodium dichromate (0.5 per cent.) was carefully poured on the gel. As the sodium salt contains two molecules of water of crystallisation, while the potassium salt is anhydrous, the 0.5 per cent. solution of the former has a slightly lower equivalent concentration than that of the latter; nevertheless, it diffuses somewhat more rapidly into the gel than the potassium salt. Apart from this, the results are indistinguishable. The molar concentrations are thus: 0.1 per cent. of crystallised lead acetate = 0.246 millimoles, 0.5 per cent. of crystallised potassium dichromate = 1.70 millimoles. The component which is to diffuse into the gel is accordingly, as is quite generally the case, in considerable molar excess. It was later found that both concentrations could be doubled, and equally good results be obtained somewhat more rapidly. In some cases the gel was made acid by the addition of one to five drops of N/1 acetic acid to 10 c.c. of gel; it is not necessary to enter into the effect of this addition beyond saying that the strata become somewhat more substantial and slightly further apart.

In the initial experiments test-tubes containing generally 10 c.c. of gel were used, and measured volumes, varying from 1 to 4 c.c., of dichromate solution were placed on top of the gel. The test-tubes were placed in the ordinary racks on the laboratory table. With this arrangement, normal stratifications were obtained only for a short distance in the top portion of the gel column; as diffusion progressed, a notable change occurred, wide bands of precipitate being formed at intervals between the normal strata (fig. 4). A striking feature of these anomalous bands is that the distance between adjoining ones *decreases* as the reaction proceeds, while the distance between the normal strata always increases. Repetition of the experiment at intervals led to substantially the same result, although the number of normal strata in later specimens decreased noticeably (fig. 5). A very curious feature was observed in the sample of agar, mentioned above, which failed to produce normal strata at all; the abnormal bands are nevertheless well marked in the uniform precipitate (fig. 6).

The first explanation of the anomaly which suggested itself was the rapid decrease in the concentration of the incoming reaction component, with the particular ratios employed, although it must be mentioned at once that no

such abnormal formations have ever been described in the case of other reactions carried out with quite arbitrary volumes of solution and gel. On the other hand it had to be considered that the concentrations used were exceptionally low compared with those employed in most other reactions; thus the lead iodide reaction gives the best results in 1 per cent. agar gel containing 2 to 4 per cent. of KI, covered with a 30 per cent. solution of  $\text{Pb}(\text{NO}_3)_2$ , *i.e.*, with molar concentrations over 500 times as great as those used in the lead chromate reaction.

To eliminate this possibility it seemed advisable to return to the original object of the investigation and to use a volume of dichromate solution so large in comparison with that of the gel that the decrease in concentration caused by the diffusion into the latter and by the reaction would be negligible. The test tubes containing the agar gel were accordingly immersed completely in dichromate solution, stirred at intervals and contained in glass jars from 300 to 1,500 c.c. capacity. All these produced very perfect stratifications of the normal type: in those containing the slight addition of acetic acid mentioned above the curious "faults" and anastomoses between adjoining strata, which are highly characteristic of agar\* show themselves strikingly (fig. 2). In some specimens this joining of adjacent strata is so regular that the whole system forms a continuous pseudo-helical surface (fig. 3).

The strata show, from the top downward, the regular increase in distance which is generally, and no doubt correctly, ascribed to the gradually decreasing concentration of the component in the gel. It seemed desirable to proceed one step further and to use both a large volume of solution and a large volume of gel, in order to study the effect of keeping the concentrations of *both* components practically constant. The simple arrangement shown in fig. 7 was used for this purpose, with various unessential modifications. A tube A, about 1 cm. bore and filled with the lead acetate—agar gel dips into a flask containing from 120 to 200 c.c. of the same gel. The top of the tube carries a funnel, B, containing about 120 c.c. of dichromate solution. As the volume in a length of 20 cm. of tube is about 15 c.c., this arrangement keeps the concentrations of both lead acetate and dichromate constant within roughly 12 per cent.

Various experiments were carried out with this arrangement, all of which again produced the anomalous phenomenon, in an accentuated form; in the majority of cases the normal stratifications were confined to the top of the gel column (fig. 8). The assumption that the constant concentration of dichromate, in the jar experiments, prevented the anomaly, was therefore rendered untenable.

\* Cf. E. Küster, 'Koll.-Zeitschr.,' vol. 18, p. 107 (1916).

At this stage it was decided to mark the progress of diffusion every twenty-four hours; the marks showing in fig. 8 were placed at 11 P.M. From this, and from numerous parallel specimens, it became quite evident that the abnormal strata were a *diurnal phenomenon*.

The only diurnal variation which suggested itself as likely to influence the phenomenon was that in temperature. In this connection it should be mentioned that the preparations were kept in two rooms with north aspect one of which was not heated while the other was warmed day and night. The temperature in the latter during the period covered by the experiments varied, roughly speaking, between 11° and 16° C., while that of the other was within 2° or 3° of the outside temperature. As each experiment extended over at least a week, and in many cases over two, the use of an efficiently stirred thermostat was inconvenient, and various forms of heat insulation were tried. The apparatus illustrated in fig. 7 was placed inside a tall cylinder filled with sawdust; in other cases only the tube A was surrounded by a wider tube, the space between the two being filled with sawdust. In other experiments test-tubes with only 2 to 4 c.c. of dichromate solution were placed in boxes filled with sawdust or shredded asbestos.

All these preparations, whether a small or large volume of dichromate solution was used, produced normal strata. Test-tubes immersed in a large volume of water, and fitted with funnels containing various volumes of dichromate solution, however, persistently produced anomalous results, although the heat insulation in this arrangement should have been equally effective as when the tubes were submerged in the dichromate solution itself.

The only possible conclusion was that the anomaly was produced by light. Dichromate solution, even at a concentration of 1 per cent. and in very moderate thickness, cuts off the rays of shorter wave-length than about 5,200 very completely, so that the test-tubes submerged in it were exposed only to the non-actinic portion of the spectrum. In all the other forms of heat insulation used light was also incidentally excluded.

This unexpected conclusion was verified by carrying out reactions in pairs of preparations, one of which was exposed to diffuse light, while the other was protected by a wrapper of thin copper or tin-foil, these materials being chosen so that variations in temperature should affect both preparations equally. The results leave no doubt regarding the effect of light. The screened specimens developed normal strata throughout, whereas those exposed to light all showed anomalies. The character of the latter varied considerably, a feature which was to be expected, since the experiments extended from March to May, and the hours of daylight as well as the intensity of the latter varied considerably over the period. In all cases,

however, the formation of normal strata was interrupted when illumination was sufficient, and their place was taken by wider bands which sometimes differed markedly in colour from the normal strata and were often perceptibly denser on the side which had been turned towards the window. In the specimens obtained later in the year normal stratification generally stopped altogether after the first forty-eight hours, and only wide diurnal bands were formed after that.

It is highly interesting that both the normal and the abnormal bands can be produced side by side in the same body of gel: two specimens showing this result are illustrated in figs. 9 and 10. The gel in both cases was contained in a cell only 4 mm. deep; one half (the left one) of the face turned towards the light was covered with black paper, while the back was completely covered, so as to prevent reflection. In both cases, the anomalous strata in the right half—that exposed to light—show very clearly. The normal strata show only slightly in fig. 9, this preparation having been made with a very small volume of dichromate solution, whereas they are clearly visible throughout the left half of fig. 10, made with a large volume of solution. The irregular appearance is generally to be observed in preparations made in very shallow cells, and may be partly due to the meniscus and partly to inequalities in the agar due to rapid cooling. The progress of diffusion in fig. 10 was marked during the first three days at 9 A.M. (full lines) and 9 P.M. (dotted lines). It is worth mentioning that this preparation was made about the middle of May, when the window, which looks almost due north, received some direct light before 7 A.M. and after 7 P.M. (summer time).

At the present stage it is impossible to suggest any explanation of this very striking effect of light, but a few possibilities, which rather readily obtrude themselves, may be ruled out. The occurrence of the phenomenon in an organic medium containing a dichromate seems to indicate a clue, but the attempt to trace the anomaly to a possible reducing action of the organic gel under the influence of light does not lead very far towards a satisfactory explanation. Such an action, though it has never been recorded with agar, is not *primâ facie* improbable, as it occurs in other carbohydrates, e.g., gum arabic. To test this point, 1 per cent. dichromate solution was allowed to diffuse into duplicate specimens of pure agar gel, i.e., without any lead salts, one specimen being exposed to light and the other covered with black paper. No difference in the rate of diffusion could be detected, nor in the appearance of the specimens: the colour in both was that of pure dichromate. Both specimens, on being slowly heated together in the water bath, "melted" at the same temperature and were equally clear.

No attempt was made to separate any possible insoluble product of reduction by filtration or sedimentation, as either would have involved keeping the agar sol at about 90° C. for some time, a procedure likely to lead to reduction by itself.

It must also be remembered that the salt present in the gel throughout is *not* the dichromate, but the lead acetate, which appears extremely unlikely to be acted on by light. Gels containing lead acetate have been repeatedly kept, fully exposed to light, for several days, have been re-melted, and have eventually produced quite normal strata in test-tubes protected from light. The dichromate only diffuses in gradually, and it is very difficult to imagine that any reaction between it and the agar, under the influence of light, could proceed nearly as rapidly as the ionic reaction between dichromate and lead acetate; in other words, the precipitate of lead chromate should be formed long before any reduction of dichromate in the reaction zone, assuming it to take place at all, would seem probable. Of course, these considerations do not exclude the possibility that some product is formed which inhibits normal stratification, and that this product diffuses in advance of the dichromate. In view of the known extreme sensitiveness of the phenomenon to exiguous amounts of admixtures, this possibility must not be neglected, although it is not capable of exact formulation at the moment.

In this connection it is of interest to observe, more especially in fig. 10, that some traces of anomalous formation can be seen in the screened (left) half of the preparation. If such an inhibiting product were formed, it would of course diffuse in all directions, and would, therefore, also reach the portion of the gel protected from light. While this cannot be overlooked, an explanation decidedly more probable seems to be that scattered or reflected light has reached the shaded portion; although the back was covered with black paper, this was naturally on the outside of the glass wall, so that reflection from the internal face was still possible.

Finally, it must be mentioned that both the normal and the anomalous phenomenon can be obtained *equally well with chromate* as with dichromate. No data on the photo-chemical behaviour of chromate-carbohydrate mixtures seem to be available, but it is known that chromate-gelatin, unlike dichromate-gelatin, is *not* sensitive to light.

In the absence of other clues it seemed possible that the size of the grains of precipitate might throw some light on the mechanism of the anomaly. Several specimens, duplicates of those illustrated, were accordingly removed from the test-tubes—which can be done conveniently, as agar does not adhere to glass—and the precipitate from various regions examined and measured

with the eye-piece micrometer. The precipitate, whatever its size, consists of roughly spherical granules, pairs of them being, very occasionally, joined to form a squat "hourglass" aggregate. As regards the size, the results are not easy to interpret. They are simplest in the specimen shown in fig. 6, which did not produce normal strata at all, but only the anomalous bands caused by light embedded in a uniform precipitate. The latter consisted of granules of remarkably uniform size,  $5\mu$  average diameter, while the bands contained equally uniform granules about  $2.5\mu$  average diameter. A duplicate of fig. 5 was also investigated, samples of precipitate being taken from three portions: A, from the first set of normal strata at the top of the gel, B from the first wide daylight band, and C from the three normal strata below this. A consisted of granules  $1.25$  to  $1.8\mu$  dia., B about  $2.5\mu$  dia., and C  $3.7$  to  $5\mu$  dia.

The latter results are difficult to interpret definitely; as regards the specimen, fig. 6, it is evident that the number of centres of crystallisation in the daylight bands is considerably greater than in the precipitate formed in the dark, or that the limit of supersaturation is altered. The question what causes this difference of course remains unanswered: a direct effect of light, in the absence of parallel cases, would have to be proved by more substantial evidence, while the formation of some reaction product which affects supersaturation or the number of nuclei is possible, but equally in need of proof. Further research must be directed to these two points, and the first step seems to be the elimination of the organic matter, by studying the formation of lead chromate, either in an inorganic gel, *e.g.*, silicic acid, or possible merely in aqueous solutions. It will also be necessary to ascertain whether the effect is peculiar to this one reaction, or whether it is of a more general nature.

In the meantime it is permissible to draw attention to one point, even in the absence of any explanation of the effect. The possible bearing of the Liesegang phenomenon on the formation of rhythmic structures in nature, and in conditions where there is no obvious rhythm or periodicity in the supply of reacting material, has been repeatedly pointed out, by Liesegang himself, by H. Bechhold, D'Arcy W. Thompson, E. Küster and others. If the diurnal change in illumination is capable of modifying profoundly the stratifications produced by a given reaction, it is evident that structures of much greater complication than those contemplated by the authors quoted may conceivably be the result of simple and non-periodic diffusion.

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